

I claim:

1. A method for identifying a sequence of nucleotides in a polynucleotide, the method comprising the steps of:
- (a) extending an initializing oligonucleotide along the polynucleotide by ligating an oligonucleotide probe thereto to form an extended duplex;
  - (b) identifying one or more nucleotides of the polynucleotide; and
  - (c) repeating steps (a) and (b) until the sequence of nucleotides is

10 determined.

2. The method of claim 1 wherein said oligonucleotide probe has a chain-terminating moiety at a terminus distal to said initializing oligonucleotide.

3. The method of claim 2 wherein said step of identifying includes removing said chain-terminating moiety and extending said oligonucleotide probe with a nucleic acid polymerase in the presense of one or more labeled chain-terminating nucleoside triphosphates.

4. The method of claim 3 futher including a step of regenerating an extendable terminus on said extended duplex.

5. The method of claim 4 wherein said oligonucleotide probe includes a subsequence of four ribonucleotides and wherein said step of regenerating includes cleaving said oligonucleotide probe with RNase H.

6. The method of claim 5 wherein said chain-terminating moiety is a 3' phosphate.

7. The method of claim 2 further including a step of capping an extended duplex or said initializing oligonucleotide whenever the extended duplex or said initializing oligonucleotide fails to ligate to said oligonucleotide probe.

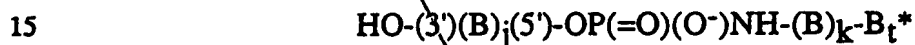
8. The method of claim 2 further including a step of regenerating an extendable terminus on said extended duplex. ✓
9. The method of claim 8 wherein said step of regenerating includes  
5 cleaving a chemically scissile internucleosidic linkage in said extended duplex.
10. The method of claim 9 wherein said chemically scissile internucleosidic linkage is a phosphoramidate.
- 10 11. The method of claim 8 wherein said step of regenerating includes enzymatically cleaving an internucleosidic linkage in said extended duplex.
12. The method of claim 11 wherein said oligonucleotide probe includes  
15 a subsequence of four ribonucleotides and wherein said step of regenerating includes cleaving said oligonucleotide probe with RNase H.
13. A method for determining the nucleotide sequence of a polynucleotide, the method comprising the steps of:
- 20 (a) providing a template comprising the polynucleotide;  
(b) providing an initializing oligonucleotide which forms a duplex with the template adjacent to the polynucleotide;  
(c) annealing an oligonucleotide probe to the template adjacent to the initializing oligonucleotide;  
25 (d) ligating the oligonucleotide probe to the initializing oligonucleotide to form an extended duplex;  
(e) identifying one or more nucleotides of the polynucleotide by a label on the ligated oligonucleotide probe; and  
(f) repeating steps (c) through (e) until the nucleotide sequence of  
30 the polynucleotide is determined.
14. The method of claim 13 wherein said oligonucleotide probe has a chain-terminating moiety at a terminus distal to said initializing oligonucleotide and wherein said method further includes a step of  
35 regenerating an extendable terminus on said oligonucleotide probe.

15. The method of claim 14 further including a step of capping said extended duplex or said initializing oligonucleotide that fails to ligate to said oligonucleotide probe.

5 16. The method of claim 14 wherein said step of identifying consists of identifying a single nucleotide of said polynucleotide.

17. The method of claim 16 wherein said step of identifying includes removing said chain-terminating moiety and extending said oligonucleotide probe with a nucleic acid polymerase in the presense of one or more labeled chain-terminating nucleoside triphosphates.

18. An oligonucleotide probe of the formula:



wherein:

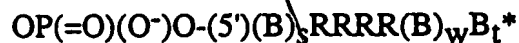
B is a nucleotide or an analog thereof;

j is in the range of from 1 to 12;

20 k is in the range of from 0 to 12, such that the sum of j and k is less than or equal to 12;

B<sub>t</sub><sup>\*</sup> is a labeled chain-terminating moiety.

25 19. An oligonucleotide probe selected from the group consisting of:



30



and

35



wherein:

B is a deoxyribonucleotide or an analog thereof;

R is a ribonucleotide;  
s is in the range of from 1 to 8;  
w is in the range of from 0 to 8, such that the sum of j and k is less than or equal to 8;  
5 B<sub>t</sub>\* is a labeled chain-terminating moiety.

Add  
A<sup>3</sup>

Add  
A<sup>4</sup>

Add  
C<sup>2</sup>

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